SI -- Preparation of nitrogen-doped carbon dots and their enhancement on lettuce yield and quality

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1. Experiments

1.1 Cytotoxicity of CDs

The MTT method was used to evaluate the toxicity by the effect of CDs on Hela cells. First, Hale cells were cultured in cell culture flasks (37°C, 5% CO_2) containing FBS medium (10%) for 8 h, followed by replacement with fresh medium. Cells were transferred to medium containing different concentrations (0, 20, 50, 100, 200 $mg \cdot L^{-1}$) and continued to be cultured for 24 h. Then the medium was discarded again, and the complete medium and MTT solution were added to continue the incubation for 4 h, and removed again. After adding dimethyl sulfoxide (150 μL) and shaking for 10 min, the absorbance value at 570 nm was measured using an enzyme marker.

1.2 Functional leaf area measurement

Weighing method was used to measure the functional leaf area of lettuce. After 14 days of N-CDs treatment, functional lettuce leaves (leaves with the largest area) were taken from each group, and after removing the veins, the total leaf weight M_1 was weighed, a flat circular piece with area S_1 was cut off at the same part of the leaf in each group, and the mass M_2 was measured after removal, and the total leaf area S_L was calculated by the following equation:

$$S_L(cm^2) = \frac{M_1 \times S_1}{M_2}$$

Where S_L is the functional leaf area of lettuce; M_1 is the total weight of leaf; S_1 is the area of the circular piece; M_2 is the mass of the circular piece.

1.3 Soluble sugar content measurement

In a 10 mL centrifuge tube containing 8 mL of 80% ethanol, lettuce 0.5 g was added, and a water bath at 80°C for 40 min, and then left to cool. Aspirate 0.2 mL of the sample extract, add 0.8 mL of ultrapure water, mix well, then add 5 mL of Anthrone reagent (100 mg Anthrone dissolved in 100 mL dilute sulfuric acid), boil the water bath for 10 min and cool down. The OD value was measured at 630 nm. The formula was calculated as follows:

$$Soluble\ sugar\ content(\mu g \cdot g^{-1}) = \frac{X \times V_1 \times n}{W \times V_2}$$

Where X is the amount of soluble sugar calculated from the regression equation (μg); V_1: the volume of sample fixation (25 mL); V_2 represents the volume of sample extract taken for the determination (0.2 mL); n represents the dilution multiple (5); W is the weight of the weighed sample (g).

1.4 Soluble protein content measurement

0.5 g of lyophilized lettuce sample was added to a 10 mL centrifuge tube, 8 mL of ultrapure water was added, and the homogenate was ground and centrifuged at 3000 rpm for 10 min. Take 0.2 mL of the supernatant in a test tube, add 0.8 mL of ultrapure water, and then add 5 mL of Kaumas Brilliant Blue G-250 solution to each test tube, shake well, and leave for 5 min. The absorbance was measured at 595 nm. The soluble protein content of the samples was calculated as follows:

$$Soluble\ protein\ content(\mu g \cdot L^{-1}) = \frac{X \times V_1 \times 5}{W \times V_2}$$

Where X is the amount of soluble protein (μg) calculated using the regression equation; V_1 is the volume of fixed volume when measuring the sample (8 mL); the dilution multiple (5); V_2 is the volume of sample supernatant added when measuring the sample (1 mL); W is the mass of

the sample weighed (g).

1.5 Vitamin C content measurement

10 mL of oxalic acid-EDTA solution was added to the centrifuge tube, weigh 0.5 g of lettuce lyophilized sample into the centrifuge tube, transfer the solution from the centrifuge tube to a 50 mL volumetric flask, and fix the volume. Pipette 10 mL of the solution into a 25 mL test tube, add 1 mL of metaphosphoric acid-acetic acid solution, 2 mL of 5% sulfuric acid. Add 4 mL of ammonium molybdate solution, shake well for 15 min and measure the absorbance value at 705 nm. The Vc content ($\mu g \cdot g^{-1}$) in a unit weight sample was calculated as follows:

$$Vc\ content(\mu g \cdot g^{-1}) = \frac{X \times V_1}{W \times V_2}$$

Where X is the amount of VC calculated from the regression equation (μg); V_1 indicates the volume of the sample fixation (50 mL); V_2 indicates the volume of the sample extract taken for the determination (10 mL); W is the weight of the sample weighed (g).

1.6 Nitrate content measurement

Weigh 1.0 g of lyophilized sample powder into a centrifuge tube, add 10 mL of ultrapure water, and boil the water bath for 30 min. After cooling, filter the extract into a 25 mL volumetric flask and rinse the residue repeatedly, and finally fix the volume to the scale. Aspirate 0.1 mL of sample solution in a test tube, then add 0.4 mL of 5% salicylic acid-sulfuric acid solution, mix well and leave it at room temperature for 20 min, then slowly add 9.5 mL of 8% NaOH solution and shake well. After shaking and cooling, the absorbance value was measured at 410 nm using blank as reference. The nitrate content ($\mu g \cdot g^{-1}$) in a unit weight sample was calculated by the following formula:

$$Nitrate\ content(\mu g \cdot g^{-1}) = \frac{X \times V_1}{W \times V_2}$$

Where X is the amount of nitrate calculated from the regression equation (μg); V_1 is the

volume of the sample fixation (25 mL); V_2 indicates the volume of the sample extract taken for the determination (0.1 mL); W indicates the weight of the sample weighed (g).

1.7 Rubisco activity determination

Reagent: 40 mmol·L^{-1} (pH 7.6) Tris-HCl buffer solution (containing 10 mmol·L^{-1} $MgCl_2$, 0.25 mmol·L^{-1} EDTA, 5 mmol·L^{-1} glutathione); 4 mM NADH + 10 mmol·L^{-1} RuBP + 100 mmol·L^{-1} phosphocreatine; ATP; glyceraldehyde -3-phosphate dehydrogenase, phosphoglycerate kinase; working solution: 22.5 mL of ATP (4 mM NADH + 10 mmol·L^{-1} RuBP + 100 mmol·L^{-1} phosphocreatine) was added, mixed thoroughly and set aside.

Take 0.5 g of lettuce lyophilized sample, add 5 mL Tris-HCl buffer solution, and perform ice bath homogenization. Centrifuge the sample at 10,000 rpm for 10 min at 4 °C. Add 6 μL of supernatant, 7 μL of glyceraldehyde-3-phosphate dehydrogenase, 7 μL of phosphoglycerate kinase and 180 μL of working solution, mix well, and measure the absorbance values at 340 nm for 20 s and 2 min 20 s. The absorbance values were recorded as A_1 and A_2, respectively. The absorbance values were recorded as A_1 and A_2, respectively, and $\Delta A=A_1-A_2$ was calculated. Rubisco activity was calculated from the following equation:

$$R(\mu mol \cdot mL^{-1} \cdot min^{-1}) = \frac{(\Delta A \times V_T)/(\varepsilon \times d) \times 10^6}{(M \times V_S/V_{TS}) \times T} = \frac{26.8 \times \Delta A}{M}$$

Where V_T is the total volume of reaction system, 2×10^{-4} L; ε is the molar extinction coefficient of NADH, 6.22×10^3 L·mol^{-1}·cm^{-1}; D is the optical diameter of 96-well plate, 0.5 cm; V_S is the volume of sample added, 0.006 mL; V_{TS} is the volume of extraction solution added, 5 mL; T is the reaction time, 2 min; M is the fresh weight of sample, g.

1.8 GS activity determination

Reagent: Extraction solution: 0.05 mol·L^{-1} Tris-HCl buffer, 2 mmol·L^{-1} Mg^{2+}, 2 mmol·L^{-1} DTT, 0.4 mmol·L^{-1} sucrose, adjusting the pH to 7.4; Reaction solution A: 0.1 mol·L^{-1} Tris-HCl buffer, 80 mmol·L^{-1} Mg^{2+}, 20 mmol·L^{-1} sodium glutamate, 20 mmol·L^{-1} cysteine and 2 mmol·L^{-1} EGTA, adjusting the pH to 7.4; Reaction solution B: 0.1 mol·L^{-1} Tris-HCl buffer, 80 mmol·L^{-1}

hydroxylamine hydrochloride, 80 mmol·L^{-1} Mg^{2+}, 20 mmol·L^{-1} glutamate sodium salt, 20 mmol·L^{-1} cysteine and 2 mmol·L^{-1} EGTA, adjusting the pH to 7.4; 40 mmol·L^{-1} ATP solution. Color development solution: 0.2 mol·L^{-1} TCA, 0.37 mol·L^{-1} $FeCl_3$ and 0.6 mol·L^{-1} HCl mixture.

A freeze-dried sample of lettuce was taken 0.5 g, added with 5 mL of extraction solution and homogenized in an ice bath. The sample was centrifuged at 8000 rpm for 10 min at 4°C, and the supernatant was removed and placed on ice for measurement. Add 70 μL of supernatant, 160 μL of reaction solution B and 70 μL of 40 mmol·L^{-1} ATP solution, mix well, and then bath for 30 min at 37°C. Add 100 μL of color development solution, mix well, let stand for 10 min, and then centrifuge at 8000 rpm for 10 min at room temperature. The absorbance value was recorded as A2, and ΔA=A1-A2 was calculated. GS activity was calculated from the following equation:

$$GS(U\cdot g^{-1}) = \frac{\Delta A \times V_T}{(M \times V_S/V_{TS}) \times 0.005 \times T} = \frac{190 \times \Delta A}{M}$$

Where V_T is the total volume of the reaction system, 0.4 mL; V_S is the volume of added sample, 0.07 mL; V_{ST} is the volume of added extraction solution, 5 mL; T is the reaction time, 30 min; M is the fresh weight of the sample, g.

Table 1

Biological significance of chlorophyll fluorescence

Parameters	Abbreviations	Biological significance
Actual photosynthetic efficiency of PS II	Y(II)	Actual light energy conversion efficiency, actual quantum yield of photosystem II
Photochemical burst	qP	The proportion of energy absorbed by photosystem II that is used to carry out photochemical reactions, reflecting photosynthetic activity
Non-photochemical burst	NPQ	The proportion of energy absorbed by photosystem II for dissipation into heat, and the ability of the plant to dissipate excess light energy into heat, i.e. photoprotective capacity
Electron transfer rate of PS II	ETR	Relative linear electron flow rate through photosystem II
Quantum yield of regulatory energy dissipation in PSII	Y(NPQ)	The part of the excitation energy absorbed by photosystem II that is dissipated into heat by a regulated photoprotection mechanism
Quantum yield of unregulated energy dissipation in PS II	Y(NO)	The part of the excitation energy absorbed by photosystem II, passively dissipated as heat and emitting fluorescence, is mainly contributed by the photosystem II reaction center in the closed state

2. Supplementary Figures

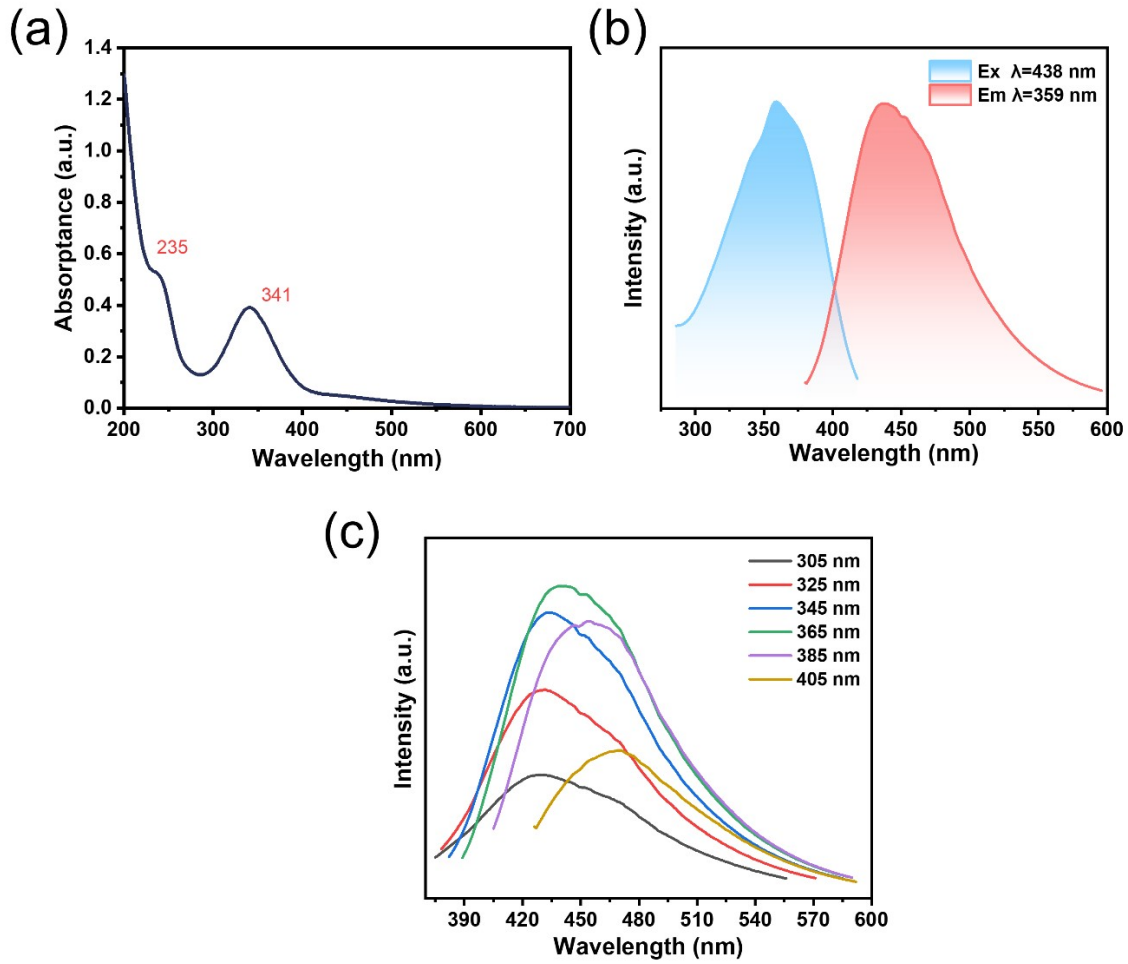

Fig.S1 (a) UV-Vis absorption spectra of N-CDs; (b) maximum FL excitation and emission spectra of N-CDs; (c) emission spectra of N-CDs with excitation wavelengths from 305 nm to 405 nm.

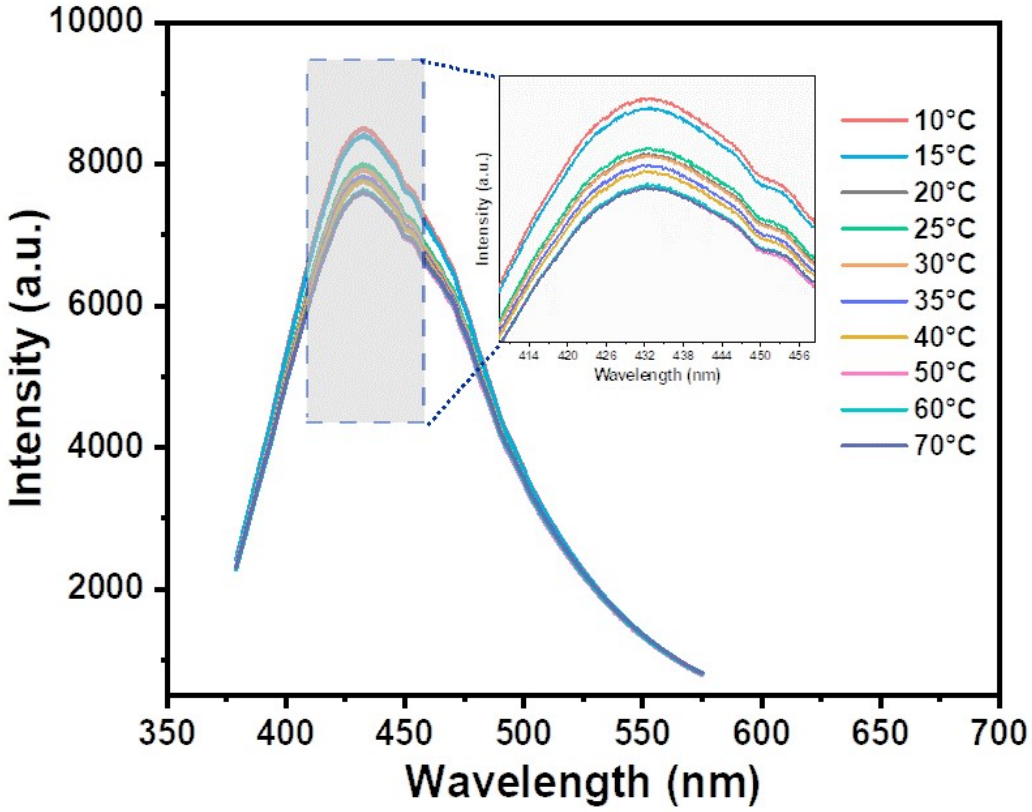

Fig.S2 Photoluminescence of N-CDs at different temperatures.

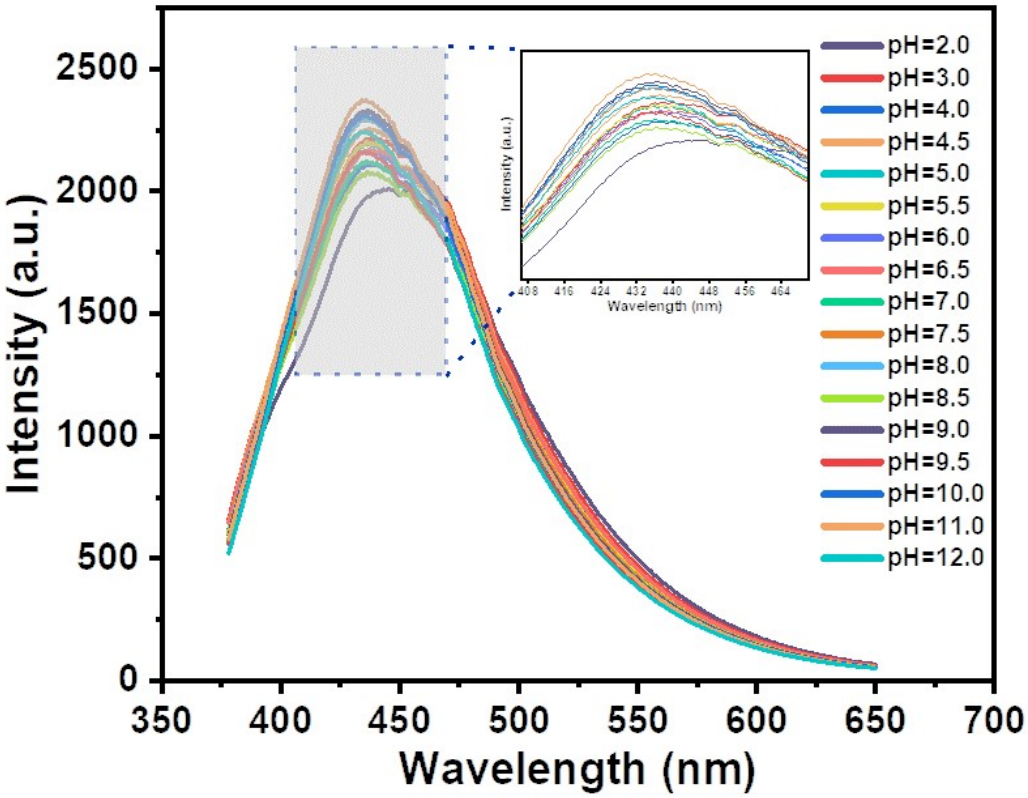

Fig.S3 Photoluminescence of N-CDs at different pH.

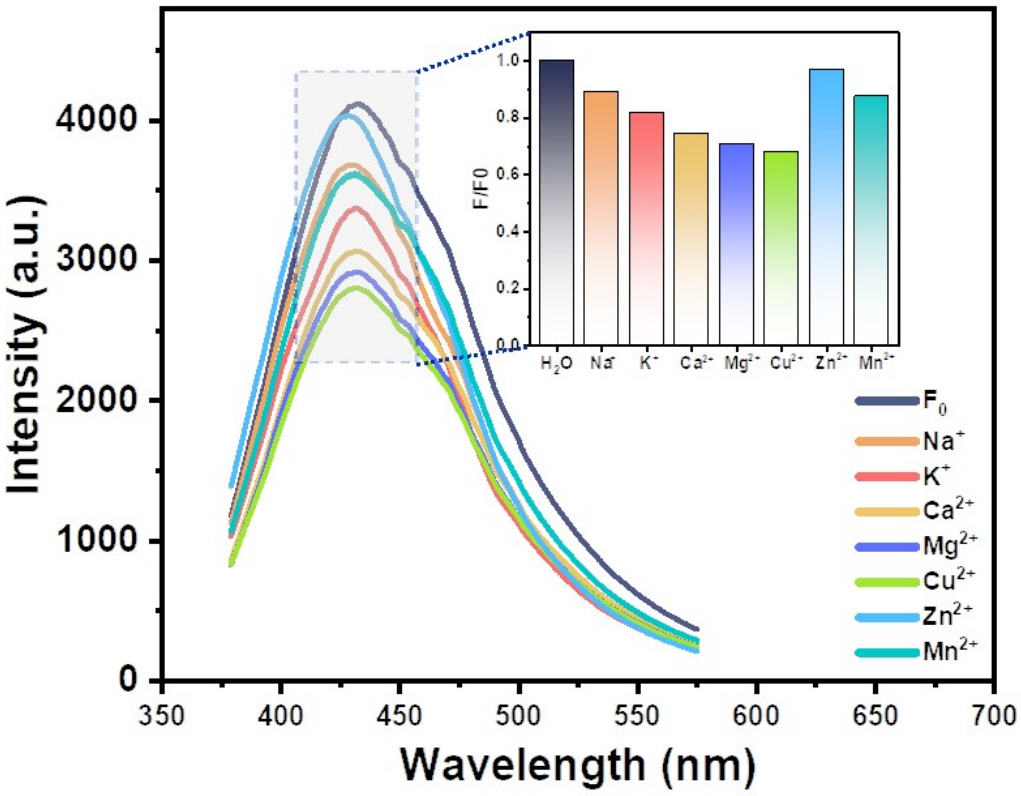

Fig.S4 Photoluminescence of N-CDs under different ionic solutions.

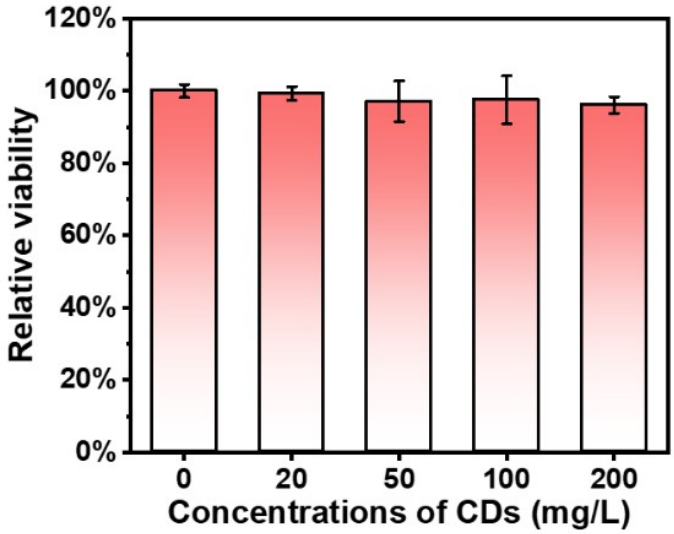

Fig.S5 Viability of Hela cells after 24 h of incubation with different concentrations of N-CDs.

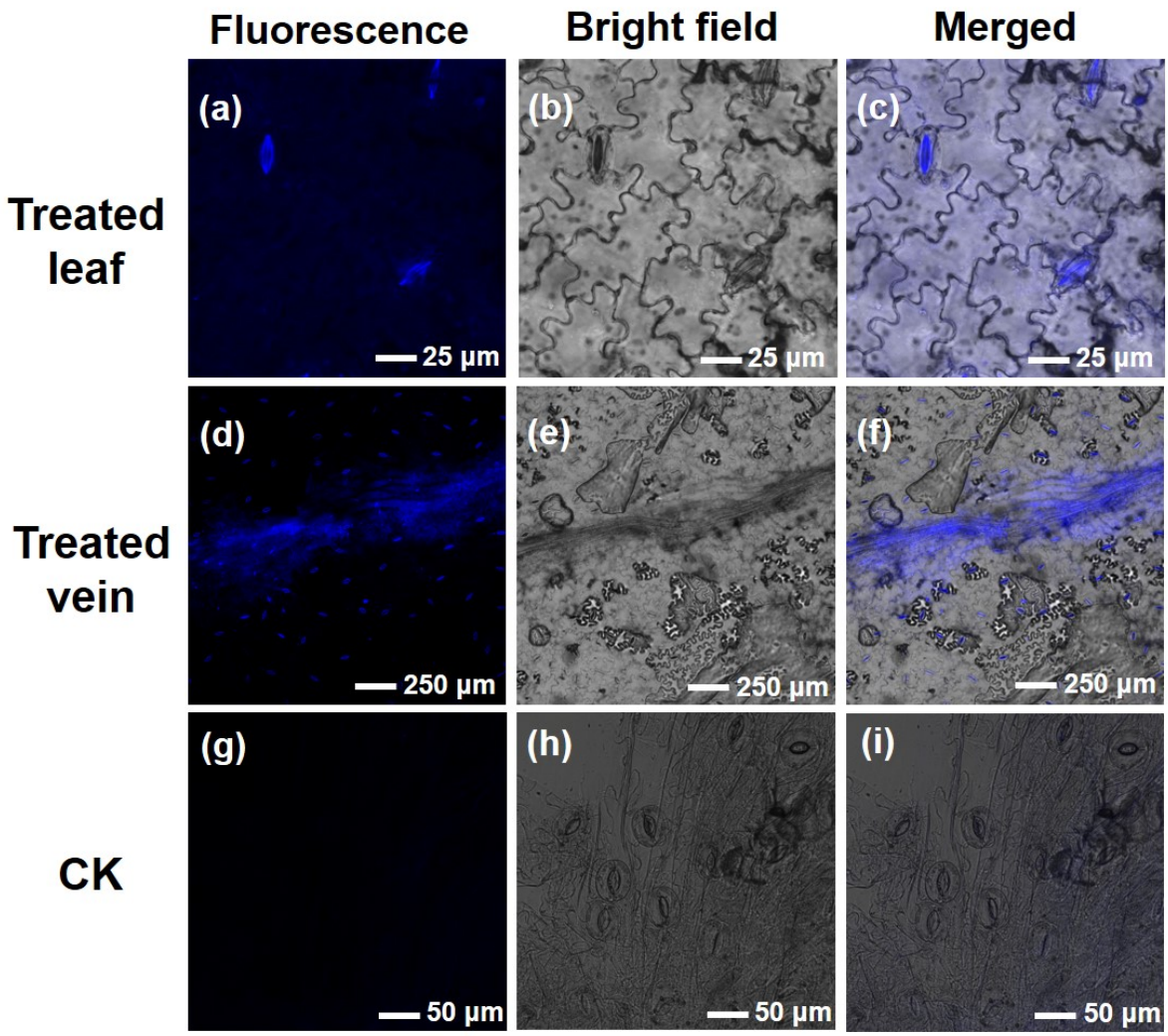

Fig.S6 (a), (d), (g) Fluorescence, (b), (e), (h) bright field and (c), (f), (i) merged images of lettuce leaves and veins in the carbon dot treatment and control groups.

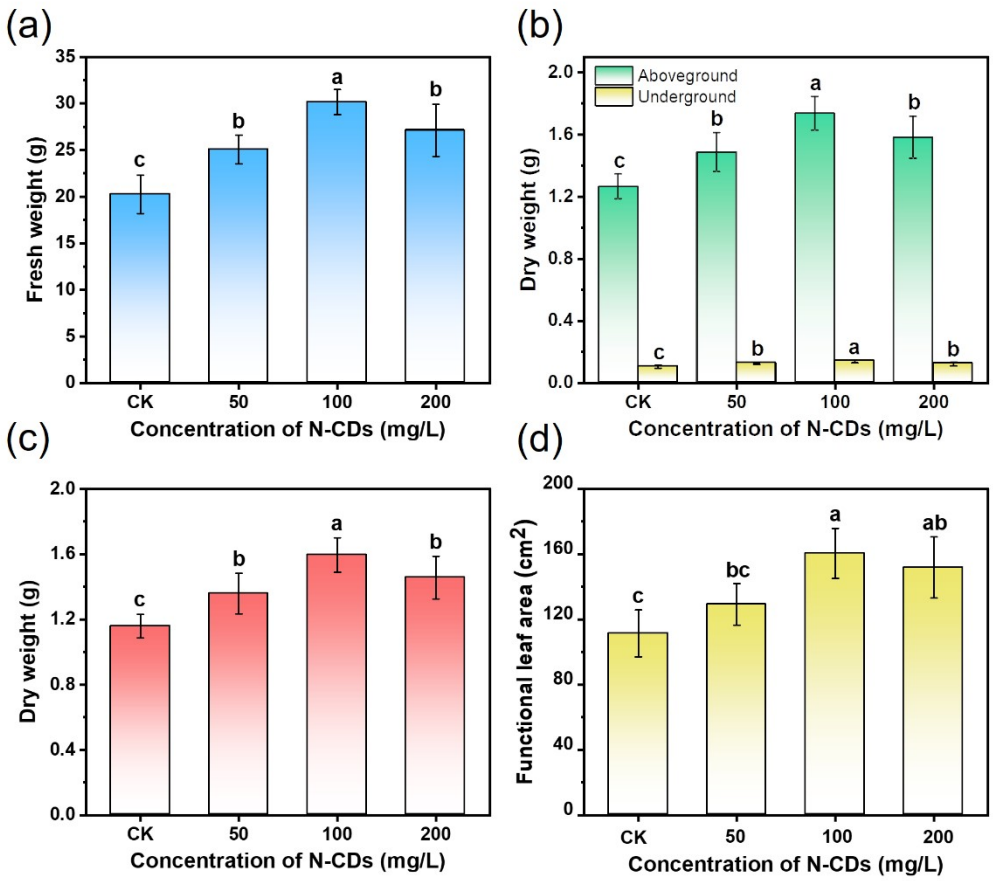

Fig.S7 (a) Total fresh weight of lettuce after 14 d of treatment with each concentration of N-CDs (0, 50, 100 and 200 mg/L); (b) aboveground dry weight and underground dry weight of lettuce; (c) Total dry weight of lettuce; (d) functional leaf area of lettuce for each group measured by weighing method. Error bars correspond to standard deviation (n = 3). Marked with different letters indicate a significant difference ($p < 0.05$).

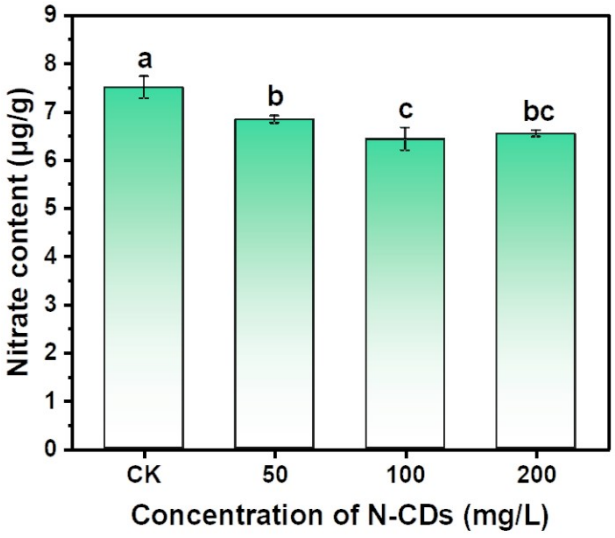

Fig.S8 Nitrate content of lettuce. Error bars correspond to standard deviation (n = 3). Marked with different letters indicate a significant difference ($p < 0.05$).

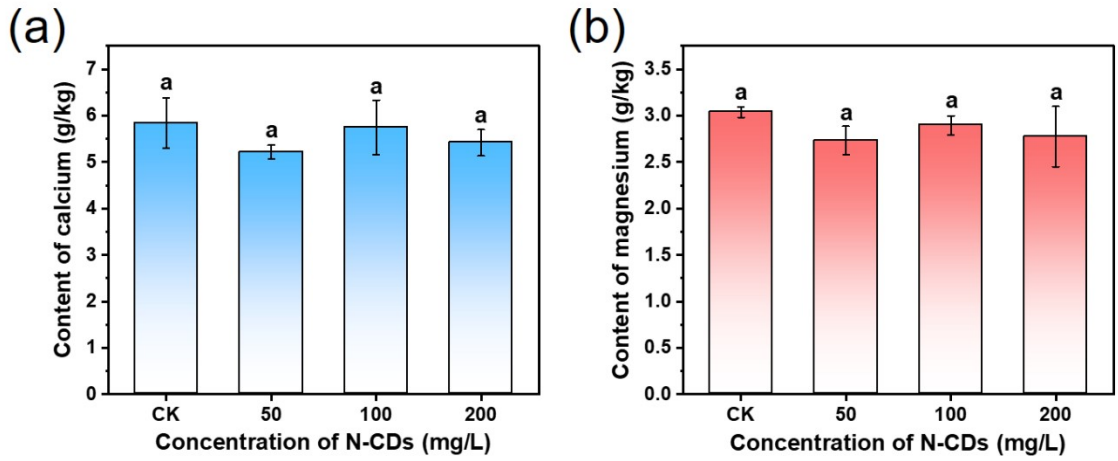

Fig.S9 Lettuce calcium(a) and magnesium(b) content. Error bars correspond to standard deviations (n = 3). Significant differences ($P < 0.05$) are indicated by different letter labels.

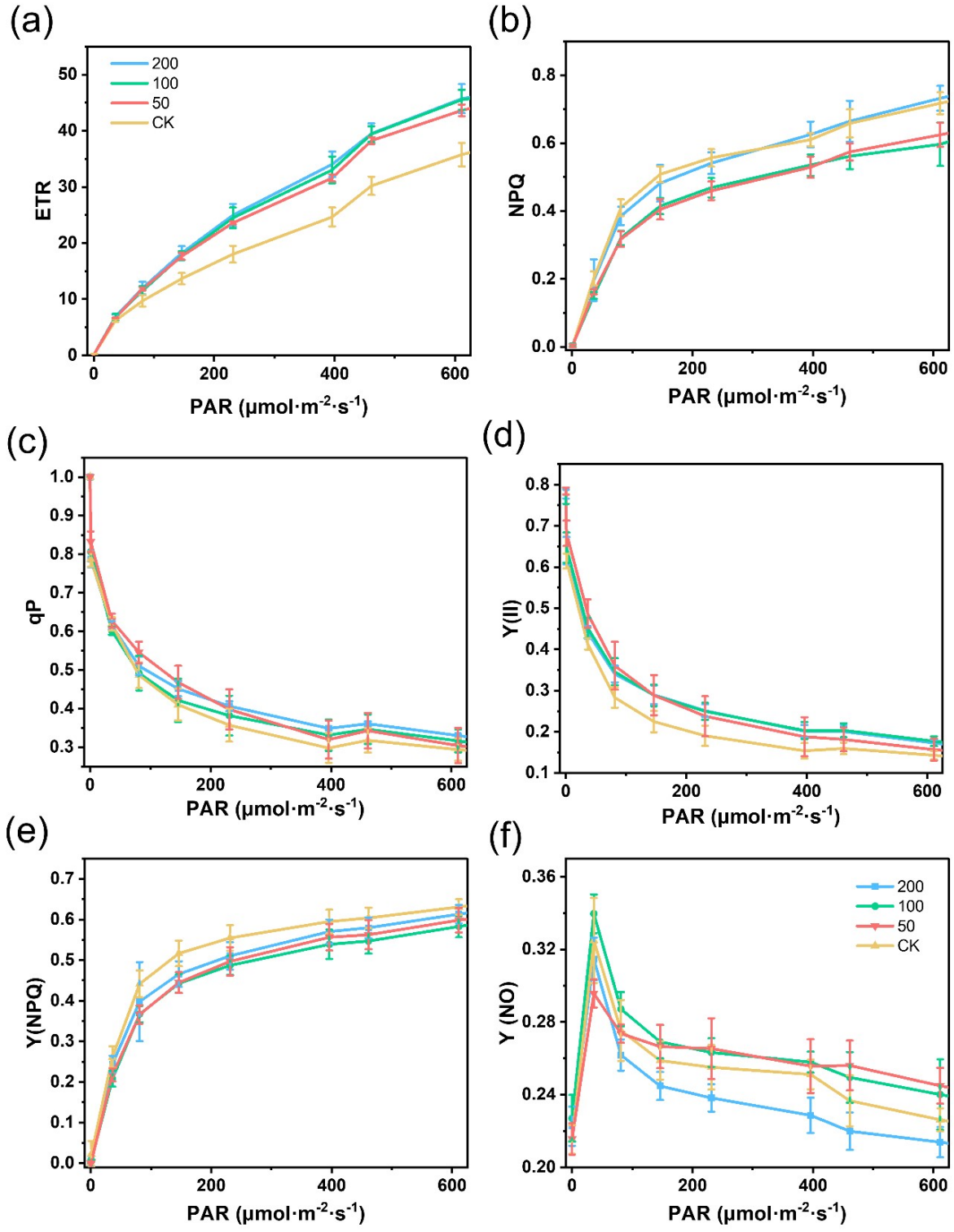

Fig.S10 (a) Relative electron transport rate (ETR) of photosystem II; (b) quantum yield of regulated energy dissipation of photosystem II; (c) photochemical quenching; (d) actual photometric quantum efficiency of photosystem II; (e) photoprotective capacity; (f) quantum yield of unregulated energy dissipation of photosystem II. Error bars correspond to standard deviations ($n \geq 4$). Significant differences ($P < 0.05$) are indicated by different letter label.